

Antioxidant Activity and Properties of Walnut Brown Seed Coat Extract

Parisa Sadighara¹, Sara Godarzi¹, Mahmoud Bahmani², Majid Asadi-Samani^{3*}

¹*Department of Environmental Health, Food Safety Division, Faculty of Public Health, Tehran University of Medical Sciences, Tehran, Iran.*

²*Biotechnology and Medicinal Plants Research Center, Ilam University of Medical Sciences, Khorramabad, Iran*

³*Student Research Committee, Shahrekord University of Medical Sciences, Shahrekord, Iran.*

*Corresponding Author: **Majid Asadi-Samani.**

Abstract: To date, there are few studies about the properties of walnut brown seed coat. The antioxidant activity and properties of walnut brown seed coat was considered in this study and the amounts of carotenoids, chlorophylla, chlorophyllb, vitamin E were determined. The antioxidant activity was determined by cupric assay, DPPH, and measurement of oxidative stability in sunflower oil against Copper sulfate. Walnut brown seed coat at 1% was effective antioxidant in comparison to BHT in inhibition of lipid peroxidation. This extract presented the highest vitamin E level (1.4 ± 0.042 mg/g). DPPH assay showed the extract had the high radical scavenging ability. The results of this study showed the walnut brown seed coat possessed strong antioxidant properties in vitro, hence it might be beneficial in human health.

Keywords: Walnut, Antioxidant activity, Vitamin E, Carotenoids, Chlorophylls.

Introduction

Lipid oxidation is a process that happens during processing and storage of lipids and lipid-containing foods. These reactions have bad effects on color, flavor, shelf life, texture and nutritional value of food products and lead to their deteriorations. Actually, the oxidative stability of fatty food depends on the balance between antioxidant and oxidizing agents.

Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are synthetic antioxidants that are used widely. Today, interest in the application of natural antioxidants, due to toxicological effects of synthetic antioxidants has increased and the consumers have tended to use natural products [1-5]. Actually, natural

antioxidants have an important role on the prevention oxidative changes. They can delay or prevent the oxidation of lipids [6].

Walnuts (*Juglans regia* L.) have great provenance for essential unsaturated fatty acids (linoleic and α -linolenic acids), tocopherols, and the potent antioxidant hormone, melatonin. The total antioxidant activity of Walnuts is due to variety of polyphenolic components [7, 8].

The previous studies showed that the plants which contain a high amount of vitamins or carotenoids possess a high level of antioxidant power [9]. This study was performed to investigate antioxidant activity and properties of walnut brown seed coat as source of natural antioxidants.

Materials and Methods

Raw Material

Walnut brown seed coat was collected, dried, and then turned into powder. The powder was extracted with 100 mL of ethanol-water mixture at 70:30 (v/v). The solution was stirred continuously for 24 h at 37 °C.

Determination of Chlorophylls

The levels of chlorophylls were determined according to previous method [10]. The solution absorbance was read on UV-260 spectrophotometer at 663 nm and 645 nm.

The amounts of chlorophyll a and Chlorophyll b were calculated according to the following formulas:

$$\text{Chlorophyll a} = (19.3 \times A_{663} - 0.86 \times A_{645}) \times V/100W$$

$$\text{Chlorophyll b} = (19.3 \times A_{645} - 3.6 \times A_{663}) \times V/100W$$

Determination of Carotenoids

The solution absorbance was read on spectrophotometer at 470 nm. Its carotenoids content was calculated on the standard curve of B carotene.

Determination of Vitamin E level

Vitamin E level was measured according to previous method [11]. Samples were exposed to Fe³⁺ solution, TPTZ and acetate buffer (pH 4). Then, the standard curve was calculated with appropriate vitamin E concentrations. The absorbance of samples was read at 595 nm wavelength.

Assay of the oxidative stability in sunflower oil model

The extract, copper sulfate (CuSO₄) and sunflower oil (without antioxidant) were mixed and shaken for 3 hours at room temperature. BHT was used as positive control. The formation of thiobarbituric acid in samples was assessed according to published method [12].

Briefly, the samples were mixed with 20% trichloroacetic acid and the mixture centrifuged. Then, thiobarbituric acid was added to the supernatant and heated. The absorbance of the supernatant was read at 532 nm. The values were expressed in μ moles of malondialdehyde.

Determination of Cupric Ion Reducing Assay (Cupric)

This method measures the cupric reducing capacity. The samples were mixed with solutions of CuCl₂ and neocuproine reagent in ammonium acetate buffer. The absorbance of solutions was read at 450 nm after incubation at 50 degrees C for 20 min [13].

Radical DPPH (1, 1- diphenyl 2-picrylhydrazyl) Scavenging Activity

The free radical scavenging activity was calculated according to scavenging activity of DPPH [12]. 3.8 cc ethanol solution of DPPH (final concentration was 0.1 mM) and 0.2 cc extract (1% extract) were mixed. The respective extraction solvent was used as negative control. The samples were shaken for 1 min and kept at room temperature in the dark for 30 min. Then, absorbance of them was measured at 517 nm against ethanol blank.

The percent of DPPH discolouration of the samples was calculated according to following formula:

$$\% \text{ discolouration} = [1 - (A_{\text{sample}}/A_{\text{control}})] \times 100$$

Results and Discussions

Safer antioxidant sources have been searched in various natural components such as different part of vegetable and fruits [14]. In the present study, the content of carotenoid pigments, level of chlorophylls and vitamin E in the walnut brown seed coat extract was evaluated and was shown in the table 1. In this study, considerable content of vitamin E was observed. The extraction rich in vitamin E and carotenoids may be good antioxidant components to prevent lipid oxidation. In the current study, the content of vitamin E was considerable amount.

Chanwitheesuk et al. reported the highest amounts were shown in the extracts of *Gymnema inodorum* and *Mentha arvensis* (9). Antioxidant compounds such as vitamins can be efficient reducing agents for inhibition of oxidative damage [15]. The results showed that walnut brown seed coat had the potential to be a good source of natural carotenoids and vitamin E. The studies have been shown that natural antioxidants have important role in immune defense system and treatment the diseases [16-28].

Table 1: Level of total carotenoids, chlorophylls and vitamin E

Total carotenoids (mg /g)	Chlorophylls(mg /g)		Vitamin E (mg/g)
	Chlorophylla	Chlorophyllb	
1.15± 0.07	2.9± 1.34	3.13± 0.4	1.4± 0.042

The level of lipid oxidation without any antioxidant was 1.12 ± 0.53 . The level of lipid oxidation was not significantly different between the group %1 extraction and the group BHT ($p > 0.05$). The level of lipid

oxidation was significantly different in %1 extraction compared to control and Vitamin C ($p < 0.05$; table 2). Therefore, this extraction has strong antioxidant to help protect from oxidative damages.

Table 2: The inhibition of lipid peroxidation by extract in sunflower oil

Component	Level of lipid peroxidation ($\mu\text{mol/ml}$)
Sunflower oil(without any antioxidant)	1.12 ± 0.53
Sunflower oil + 0.05% extraction	0.37 ± 0.1
Sunflower oil + 1% extraction	0.21 ± 0.03
Sunflower oil + 2%BHT	0.25 ± 0.06
Sunflower oil +2% Vitamin C	0.6 ± 0.06

In our finding, total antioxidant capacity was investigated by two different methods: DPPH and cupric ion reducing capacity assay (Cupric assay). The antioxidant activity was expressed as vitamin C equivalent (mg 100 /g dry matter). In DPPH

assay, antioxidant activity considered as percentage of inhibition (%IP) and was calculated (Table 3). DPPH radical scavenging activities of this extraction were high (more than %90).

Table 3: Level of antioxidant activity

The antioxidant activity assay	DPPH % IP	Cupric assay
Mean±SD	92.8 ± 0.4	107 ± 24

DPPH is widely used to assay the free radical spaces scavenging effect of antioxidants. DPPH is stable free radical at room temperature. The results from DPPH assay suggest that walnut brown seed coat possessed high radical scavenging ability.

Lipid oxidation occurs in food systems. The antioxidants should be used in foods to prevent lipid peroxidation. The oxidative stability was assayed by measuring content of malondialdehyde (MDA). The significant increase in MDA in control groups and a significant decrease in %1 extraction suggest that walnut brown seed coat prevented oxidative stress.

The antioxidant activity is contributed by the active compounds present in plants.

Natural antioxidant substances are confirmed to be safe and are seen as more acceptable than the synthetic antioxidant [9]. The pervious study reported that tea extraction (0.03%) similar to BHT (0.02%) and extract of *Melissa officinalis* at 620 ppm was as efficient as BHA at 200 ppm in preventing lipid oxidation [2]. In present study walnut brown seed coat extract (%1) was similar to BHT (2%).

These results suggest that the antioxidant power of walnut brown seed coat may be attributed to vitamin E. Vitamin E is one of the best scavenging agents for singlet oxygen, and can act as a chain-breaking antioxidant. Collectively, lipid oxidation produces genotoxic agents and changes the quality and nutritional components of foods. Therefore, natural antioxidants can be used for reducing lipid oxidation in foods.

References

1. Rafieian-Kopaei M (2015) Natural sources of vitamin D. *J Parathyroid Dis.* 3(1):10-11.
2. Rafieian-Kopaei M, Baradaran A (2013) Plants antioxidants: From laboratory to clinic. *J Nephropathol.* 2(2): 152-3.
3. Kafeshani M, Ginger (2015) Micro-inflammation and kidney disease. *J Renal Endocrinol.* 1:e04.
4. Mohammadparast V (2016) Antioxidant efficacy of *Hibiscus esculentus*. *Front Biomed.* 1(1):e04.
5. Nasri H, Abedi-Gheshlaghi Z, Rafieian-Kopaei M (2016) Curcumin and kidney protection; current findings and new concepts. *Acta Persica Pathophysiol* 1(1):e01.
6. Rouhi-Boroujeni H, Mosharraf S, Gharipour M, Asadi-Samani M, Rouhi-Boroujeni H (2016) Antihyperlipidemic effects of sumac (*Rhus coriaria* L.): Can sumac strengthen anti-hyperlipidemic effect of statins. *Der Pharm Lett.* 8(3): 143-7.
7. Akbari V, Heidari R, Jamei R, Asadi-Samani M (2015) Determination of fatty acid compositions, and pellicle antioxidant properties of different Persian walnut genotypes. *J Shahrekord Univ Med Sci.* 17(1):33-40.
8. Grace MH, Warlick CW, Neff SA, Lila MA (2014) Efficient preparative isolation and identification of walnut bioactive components using high-speed counter-current chromatography and LC-ESI-IT-TOF-MS. *Food Chem.* 158:229-8.
9. Chanwitheesuk A, Teerawutgulrag A, Rakariyatham N (2005) Screening of antioxidant activity and antioxidant compounds of some edible plants of Thailand. *Food Chem.* 92(3):491-7.
10. Misyura M, Colasanti J, Rothstein SJ (2013) Physiological and genetic analysis of *Arabidopsis thaliana* anthocyanin biosynthesis mutants under chronic adverse environmental conditions. *J Exp Bot.* 64(1): 229-40.
11. Shah AA, Khand F, Khand TU (2015) Effect of smoking on serum xanthine oxidase, malondialdehyde, ascorbic acid and α -tocopherol levels in healthy male subjects. *Pak J Med Sci.* 31(1):146-9.
12. Upadhyay R, Chaurasia JK, Tiwari KN, Singh K (2014) Antioxidant property of aerial parts and root of *Phyllanthus fraternus* Webster, an important medicinal plant. *Sci World J.* 2014.
13. Apak R, Guclu K, Ozyurek M, Celik S (2008) Mechanism of antioxidant capacity assays and the CUPRAC (cupric ion reducing antioxidant capacity) assay. *Microchim Acta.* 160 (4): 413-9.
14. Sadighara P (2012) Egg yolk: A model for screening compounds with potent antioxidants in food stuff. *Comp Clin Pathol.* 21(6):1751-2.
15. Ghatreh-Samani M, Esmaeili N, Soleimani M, Asadi-Samani M, Ghatreh-Samani K, Shirzad H (2016) Oxidative stress and age-related changes in T cells: is thalassemia a model of accelerated immune system aging? *Cent Eur J Immunol.* 41(1):116-24.
16. Hajian S (2015) Positive effect of antioxidants on immune system. *Immunopathol Persa.* 1(1):e02.
17. Khodadadi S, Rafieian-Kopaei M (2016) Herbs, health and hazards; a nephrology viewpoint on current concepts and new trends. *Ann Res Antioxid.* 1(1):e05.
18. Dehghan Shahreza F (2016) *Hibiscus esculentus* and diabetes mellitus. *J Nephropharmacol.* 5(2):104-5.
19. Baradaran A (2017) Herbal antioxidant to ameliorate vascular biology. *Angiol Persica Acta.* 2(1):e01.
20. Rafieian-Kopaei M (2013) Medicinal plants for renal injury prevention. *J Renal Inj Prev.* 2(2):63-5.
21. Nasri H (2016) Improving the nephrotoxicity of cyclosporine; the role of herbal drugs. *Toxicol Persa.* 1(1) 05.

22. Bahmani M, Asadi-Samani M (2016) Native medicinal plants of Iran effective on peptic ulcer. *J Inj Inflamm* 1(1): e05.
23. Nasri P (2017) Cancers and herbal antioxidants. *Front Biomark*. 2(1):e01.
24. Nasri H (2017) Herbs and hazards; administration of herbal drugs in maintenance hemodialysis patients. *Ann Res Dial*. 2(1): e01.
25. Raeisi E, Shahbazi-Gahrouei D, Heidarian E (2016) Pineapple extract as an efficient anticancer agent in treating human cancer cells. *Front Cancers*. 1(1):e03.
26. Nasri H (2017) Herbal drugs; from molecular studies to bedside investigations. *Aria J Front Biochem*. 2(1): e01.
27. Asadi-Samani M, Kooti W, Aslani E, Shirzad H (2016) A systematic review of Iran's medicinal plants with anticancer effects. *Journal of Evid Based Complementary Altern Med*. 21(2):143-53.
28. Baradaran A (2017) Administration of herbal drugs in geriatric individuals; trends on its helps and hazards. *Geriatr Persia*. 1(1): e01.
29. Dudonne S, Vitrac X, Coutiere P, Woillez M, Merillon JM (2009) Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. *J Agric Food Chem*. 57 (5):1768-74.